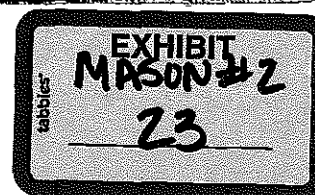


EXHIBIT 603.23



CHAPTER 6

Interpretation of Postmortem Drug Levels

Graham R. Jones, Ph.D., DABFT

Office of the Chief Medical Examiner, Edmonton, Alberta, Canada

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6.1 INTRODUCTION

In the early to mid-1900s, the practice of forensic toxicology was relatively limited in scope. Certainly, toxicologists could determine blood alcohol and a limited number of drugs with accuracy approaching that of today. However, the toxicological investigation was different in at least two respects. First, the sophistication of testing for drugs was limited, primarily relying on the efficiency of extraction techniques, followed by gravimetric and later spectrophotometric analysis. Second, with the exception of alcohol and a relatively limited number of drugs or poisons (e.g., salicylate, barbiturates, arsenic, heavy metals), there was a very limited database of reference drug concentrations available. The interpretation of quantitative results relied very heavily on the history and circumstances of the case, including the police investigation, witness accounts, and autopsy findings.

The development of gas chromatography (GC) and high-performance liquid chromatography (HPLC) during the early 1970s had a major influence on the development and growth of pharmacokinetics and therapeutic drug monitoring. As a result, the kinetics of drug absorption, distribution, metabolism, and excretion in clinical patients was easier to understand and predict. This coincided with a vast increase in the range of pure pharmaceuticals available, many of which were of lower absolute dosage compared with those previously available, for example, the replacement of barbiturates with low-dose benzodiazepines. It was logical that toxicologists started to use the pharmacokinetic data gained from living patients to interpret postmortem blood concentrations, for example, to predict whether a given blood drug concentration was "in the therapeutic range," whether the blood level was "fatal," or even to predict the amount ingested prior to death. Experience has since shown that postmortem drug concentrations must be interpreted from a perspective very different from those in living patients. Many processes occur after death that can change drug and alcohol concentrations, sometimes to a very large extent.

The period of enthusiasm in the late 1970s and 1980s has given way to the realization that there are many unique aspects of postmortem toxicology that must be considered when interpreting analytical results. It is no longer acceptable to interpret postmortem toxicology results from tables of so-called therapeutic, toxic, and fatal ranges, without taking into consideration the medical history, the immediate circumstances of the death, and the various processes that can affect drug concentrations both before and after death. It is probably fair to say that many toxicologists and pathologists are less confident about interpreting postmortem drug concentrations today — and with good reason — than they may have been 10 to 20 years ago.

It is important to remember that there are no "absolute" rules for the interpretation of toxicology results. The more information that is available to, and considered by, the interpreter, the more likely are the conclusions reached to be accurate. In the courtroom, lawyers, judges, and jurors often view all science, including the forensic subspecialties, in absolute terms. Certainly, if the toxicologist does his or her job properly, the laboratory findings will have the required accuracy. However, the subsequent interpretation is in part based on the scope of the toxicology testing (not least including the range of specimens tested), in part on the quantitative results, and perhaps, most importantly, on the history and circumstances surrounding the death. Attempts to interpret toxicology findings solely on the basis of so-called normal or reference ranges are irresponsible.

It is not the purpose of this chapter to teach anyone *how* to interpret postmortem drug concentrations, but rather to outline some of the pre-mortem and postmortem factors that should be taken into account when doing so.

6.2 GENERAL CONSIDERATIONS

6.2.1 The Analytical Result

It should be obvious that the interpretation of any toxicology test result will be no more reliable than the analytical result itself. The interpreter must be satisfied that the analysis is sufficiently accurate for the purpose, or at least know the limitations of the testing. Was the standard material used to prepare the calibrators pure and correctly identified? For example, was the salt or water of crystallization properly taken into account? Was the calibration properly prepared and valid in the range where the specimens were measured? Was the assay adequately verified by quality control samples? Was the assay sufficiently specific? Could endogenous substances or other drugs or metabolites have interfered with analysis of the specimen, either by obscuring the target analyte or by increasing the apparent concentration? If the specimen was analyzed only once, what was the potential for accidental contamination? Was there a matrix effect? For example, was recovery of the drug from the specimen the same, relatively, as from the calibrators? Using similar matrix calibrators (e.g., blood) is not necessarily a guarantee of that since postmortem blood, by its nature, is variable from case to case, or even from site to site within the same cadaver. The extraction efficiency of drug or metabolite or internal standard from animal or outdated blood bank blood may sometimes be markedly different from decomposed case blood. Although it is practically impossible to know the "absolute" or true concentration of drug in a postmortem specimen, the degree of confidence increases with the specificity of the analysis, with replication, or in some cases by applying multiple analytical methods of different physical or chemical principles.

The use of GC/mass spectrometry with multiple ion monitoring and stable isotope (e.g., deuterated) labeled internal standards will usually provide a higher degree of confidence in the accuracy of the analytical result than, say, use of an immunoassay procedure. The completeness of the analysis should also be considered. It is never possible to test for every single drug during routine screening tests. However, a careful review of the medications or other potential poisons available to the deceased should assist the laboratory in determining whether any of these substances would have been detected if present in significant concentrations.

6.2.2 Postmortem Specimens

Relying on a toxicology result from a single specimen can be misleading because of the postmortem changes that can occur. The most commonly used specimen, blood, is not a homogeneous fluid. It is good forensic practice to have multiple specimens available, or at least blood specimens from different sites in the body, because of the potential difficulties in interpreting postmortem toxicology results.

6.2.2.1 Blood

The concentrations of many drugs are affected by postmortem redistribution through the vascular system from the major organs, by direct postmortem diffusion from organ to organ, and sometimes by incomplete distribution. Sedimentation of blood after death may also affect the drug "blood" concentration obtained. For some drugs the distribution between blood and plasma is markedly uneven during life. However, toxicologists should be cautious about applying factors to "correct" for blood:plasma distribution unless it is known that the distribution is maintained after death. It may be found that the blood:plasma distribution that exists during life, due to active processes, decays after death occurs, for example, due to changes in pH and, therefore, protein binding.

Toxicologists should be cautious about inferring the exact source of a blood specimen from the labeled description. Blood, simply labeled as such, could come from almost anywhere, even

collected as pooled blood at the scene. Most toxicologists and pathologists are well acquainted with the widely discouraged practice of drawing blood by a "blind stick" through the chest wall. Although such blood may be labeled as "heart blood," it may contain pericardial fluid, or worse, may be from the pleural cavity, and therefore potentially be contaminated by gastric contents, particularly if the death was traumatic or decomposition severe.¹ Even blood drawn from the "heart" after opening the body cavity at autopsy may contain blood from a number of sources. So-called "heart" blood may contain blood from one or more of the cardiac chambers — the ventricles and atria. However, it may equally contain blood that has drained from the pulmonary vein and artery (and hence the lungs), from the inferior vena cava (and hence from the liver), and from the aorta and subclavian veins. As a result, so-called heart blood is potentially one of the most nonhomogeneous specimens in the body. As described later, postmortem redistribution and other factors can cause the concentrations of many drugs to vary markedly from site to site.²⁻⁴ Even drug concentrations in blood drawn from the same site, but simply placed into different collection vials, can also sometimes differ by severalfold.

It is generally recommended that to avoid the effects of postmortem redistribution or diffusion from the major organs, femoral blood should be sampled wherever possible. While this is certainly a good practice, interpreters should be cautioned that there is no such thing as "pure femoral blood": it is simply blood drawn from the site of the femoral vein. Certainly, if the proximal part of the femoral vein is ligated prior to sampling, it is likely that much of the blood will be "peripheral" and therefore relatively uncontaminated by blood from the major organs. However, this is rarely the case. Femoral blood is typically drawn by a "stick" to the unligated femoral vein in the groin area, such that blood will be drawn from above and below the site of sampling. If the volume drawn is relatively small (e.g., 2 to 5 mL), it is unlikely that much blood will be drawn down from the central body cavity. However, with some skill, it is often possible to draw 50 mL or more of blood from a "femoral stick." Even with a limited knowledge of anatomy, it does not require much thought to realize that at least some of this blood will have been drawn down from the inferior vena cava, and hence from the liver. An alternative sampling technique is to cut the iliac vein at the side of the pelvis during autopsy, and only sample blood that is massaged out from the femoral vein directly into a test tube. Even if such a procedure ensures that the collected blood is from the femoral vein, some postmortem changes may just as well have happened in this blood, too, e.g., diffusion from vessel walls and skeletal muscle. Since blood concentrations of some drugs have the potential for marked postmortem change, it is good practice to analyze blood obtained from more than one site, plus tissue or other specimens where this may be useful.

6.2.2.2 Vitreous Humor

Vitreous humor, although limited in volume (e.g., 3 to 6 mL), is an extremely useful specimen. It has been used for years to verify postmortem blood concentrations of ethanol, since postmortem fermentation does not occur to any significant extent in the eye. However, vitreous humor has also been useful for a number of drugs. For example, it is well known that digoxin concentrations will rise after death in cardiac blood, due to postmortem redistribution from myocardial tissue, and possibly other organs. Consequently, vitreous digoxin concentrations are more likely to reflect those in ante-mortem plasma.⁵ Vitreous humor has been used to analyze a large number of other drugs, including barbiturates, cocaine, morphine, tricyclic antidepressants, and benzodiazepines.⁶⁻¹⁰ However, interpretation of vitreous drug concentrations is difficult, in part because very few studies have been published that relate blood concentrations to those in vitreous humor, and in part because the large *ad hoc* data on vitreous drug concentrations is fragmented in innumerable case reports. In general, however, those drugs that tend to be somewhat hydrophilic at physiological pH (e.g., digoxin, benzoylecgonine, acetaminophen, salicylate) are more likely to have concentrations approaching those in blood or plasma, than those drugs that are either highly protein bound (e.g., tricyclic antidepressants) or highly lipophilic (e.g., benzodiazepines). In fact, a significant negative

correlation between the vitreous:blood concentration ratio and the degree of protein binding of different drugs has been reported.¹¹

Because the eye is remote from the central body cavity and the abdominal organs, it has been suggested that vitreous may be a useful fluid for the determination of drugs that are subject to postmortem redistribution. That may hold true for many drugs such as digoxin. However, others have shown that some drugs, notably cocaine, may increase in concentration in the vitreous humor after death.⁹ Postmortem diffusion of drugs to the vitreous from the brain, particularly in bodies lying in a prone position for an extended time, may be a possible source of error, and warrants systematic studies.

6.2.2.3 Liver

Many toxicologists rank the liver second only after blood in importance as a specimen of interpretive value in postmortem toxicology. It is particularly valuable for the tricyclic antidepressants and many other drugs that are very highly protein bound. It is useful for the phenothiazine neuroleptics which have a very large dosage range, and hence range in "therapeutic" blood concentrations. Liver tissue is also of value for interpreting postmortem concentrations of many other drugs where a sufficiently large database has been established, and particularly where blood is not available due to severe decomposition, fire, or exsanguination.

One other aspect of liver drug concentrations should be considered. It is known that postmortem diffusion from the stomach may artifactually elevate concentrations of the drug proximal to the stomach — for example, after an overdose, where both the concentration and absolute amount of drug in the stomach are high.^{12,13} However, little appears to have been done to assess the kinetics of drugs in the liver after therapeutic doses. For example, common sense would suggest that drug concentrations in the liver, and particularly those that are strongly protein bound, would increase dramatically in the period after a dose was taken, compared with that at steady state. This might be particularly important for drugs with a relatively long half-life and that are often taken in single nighttime doses, or divided with a large portion of the dose at night. As for other specimens, liver concentrations are extremely valuable for assessing the role of many drugs in a death, but only in conjunction with other analytical findings and history.

6.2.2.4 Gastric Contents

Interpretation of the analytical findings of drugs in the gastric contents is largely dictated by common sense. It is the *amount* of drug or poison remaining in the gastric contents that is important: the concentration of the drug is generally of far less importance. The tricyclic antidepressants offer a good example. Most forensic toxicologists regard total tricyclic concentrations greater than 2 to 3 mg/L, even in postmortem "cardiac" blood, as at least potentially toxic or fatal. So what does a gastric tricyclic concentration of 1500 mg/L mean? The answer is, on its own, not much, except that the person may have consumed his or her medication a relatively short period prior to death. For example, 200 mg amitriptyline at night is a fairly common dosage. If the gastric volume was, say, 120 mL, then 1500 mg/L would be completely consistent with the person taking the normal dosage just prior to death — probably from unrelated causes. However, if in our example the gastric volume at autopsy were 900 mL, then a concentration of 1500 mg/L would calculate out to 1350 mg/900 mL in the stomach, and therefore almost certainly consistent with an overdose.

Conversely, a relatively low absolute amount of drug in the gastric contents, with or without a high concentration, does not rule out the possibility of an overdose. Numerous case histories have shown that it may take several hours for an individual to die from an intentional overdose, depending on the exact drugs or poisons ingested, the amounts, co-ingestion of alcohol, general state of health, and age. It is not unusual for people to die from an oral overdose with less than a single therapeutic dose remaining in the stomach, notwithstanding the fact that an overdose of drugs can be irritant

to the stomach lining and therefore delay gastric emptying. Extensive vomiting before death can also reduce the amount of drug remaining in the stomach at the time death occurs.

Two other aspects of "gastric toxicology" should be mentioned. The simple presence of a drug in the gastric contents does not necessarily mean that the drug was recently consumed, or even prove that the drug was taken orally. Most drugs will be re-excreted into the gastric contents through the gastric juice, maintaining an equilibrium between the gastric fluid and the blood. This is especially so for drugs that are basic (alkaline) in nature. This can readily be demonstrated where it is known that a drug has only been administered intravenously under controlled conditions, and yet can be found later in small concentrations in the gastric contents. The same phenomenon can be seen with drug metabolites where, invariably, concentrations can be found in the gastric fluid. While it could be argued that microbial metabolism could have occurred in the stomach, it is more likely that the majority of the metabolites found were secreted into the stomach via the gastric juice. Conversely, the presence of "ghost" tablets in gastric contents has been reported for at least one type of slow-release analgesic, where overdose or abuse was not suspected. Apparently, the wax-resin matrix of these sustained release tablets may remain in the gastric contents long after the active ingredient has diffused out.¹⁴

More commonly, significant amounts of conglomerated, unabsorbed tablet or capsule residue can be found in the stomach many hours, or even a day or two, after a large overdose was consumed. These masses can occur after overdoses where large amounts of capsules or tablets may form a gelatinous mass, which is not readily dissolved or broken up, and which may lie slowly dissolving; they are called bezoars.¹⁵ While the term can apply to unabsorbed masses of almost anything (e.g., hair balls), it is also applied to unabsorbed drug formulations. They occur, at least in part, because gastric emptying time is delayed significantly by irritants, including large amounts of undissolved drug residue. However, the phenomenon is also occasionally seen in patients where overdosage is extremely unlikely (e.g., controlled setting such as a hospital or nursing home), but where several unabsorbed tablets may be recovered from the stomach. This is more likely to occur where enteric-coated tablets are involved, which do not dissolve in the stomach, but may stick together to form a small mass of tablets. It is also more likely to happen in elderly individuals, or in other patients where gastric motility is abnormally slow.

6.2.2.5 Urine

It is almost universally accepted that, with few exceptions, there is very little correlation between urine and blood drug concentrations, and even less correlation between urine drug concentrations and pharmacological effect. So many factors affect urine concentration, such as fluid intake, rate of metabolism, glomerular clearance, urine pH, and the times of voiding relative to the dose, that any attempt to predict or even estimate a blood concentration from a urine concentration is pure folly. As always there are some exceptions. Urine alcohol concentrations can be used to estimate the approximate blood alcohol concentration, but only if the bladder is completely voided and the measurement made on the second void. Estimates of the body burden of some heavy metals are still made on 24-h urine collections.

6.2.2.6 Brain

The brain is the primary site of action of many forensically important drugs, such as the antidepressants, benzodiazepines, and narcotics. It is potentially a very useful specimen for the measurement and interpretation of drugs because it is remote from the stomach and other major organs in the body and would not be expected to be affected by postmortem diffusion and redistribution. However, although drug concentration data in brain tissue are not hard to find in the literature, it is largely fragmented into innumerable case reports that seldom specify what anatomic

region of brain tissue was analyzed. The brain is an anatomically diverse organ such that concentrations of many drugs vary significantly from one region to another — up to about twofold.^{2,4}

6.2.2.7 Other Soft Tissues

Most of the major organs such as the kidneys, lungs, spleen, and myocardial tissue have at some time been analyzed to estimate the degree of drug or poison exposure. However, for most drugs, adequate reference databases are not available in the literature, so the interpretive value of these measurements may be limited. Skeletal muscle has the potential to be one of the most useful specimens for drug or poison determination, particularly where the body is severely decomposed, or where postmortem redistribution or diffusion might affect measurement in blood or other organs. The problem is one of obtaining sufficient reference values for that drug in skeletal muscle in order to make a confident interpretation. Some studies have been published, but data are scattered and incomplete.^{2,4,16}

The potential usefulness of bone marrow for the determination of both drugs and alcohol has been explored.¹⁷⁻¹⁹ For drugs and other poisons at least, this could be very useful in cases where severe decomposition, fire, or the action of wild animals has made the major organs unavailable, but where bone marrow can still be harvested and analyzed. As for many other specimens, the problem is again one of establishing an adequate and reliable database of reference values.

6.2.2.8 Other Fluids

Bile has been used for decades as one of the primary specimens analyzed in the forensic toxicology laboratory, but mainly for the detection and measurement of morphine. However, the usefulness of bile has decreased in the past few years as sensitive immunoassays and mass spectrometry-based assays have been developed for whole blood. For most drugs, including morphine, the interpretive value of bile is limited. Biliary drug concentrations may also be influenced by postmortem diffusion from the liver and the stomach.

Cerebrospinal fluid (CSF) is also a potentially useful specimen for the measurement and interpretation of drugs, since it is the fluid that "bathes" the central nervous system, the brain, and spinal cord. Its limitation lies mainly in the fact that it is often more difficult to collect than blood postmortem, and as for many other specimens, there is a very limited database of reference values. As for the vitreous, drugs that are highly protein bound or those that are lipophilic will tend to have significantly lower concentrations than in the blood.

6.2.2.9 Injection Sites, Nasal Swabs

Suspect injection sites are periodically excised and submitted for analysis, to support evidence of that route of administration. Certainly, it is not difficult to perform such analyses. However, the simple qualitative detection or even quantitative measurement of a drug in a piece of skin is evidence only that the drug was taken or used, not that it was necessarily injected, let alone at that site. Sometimes it is forgotten that most drugs are distributed throughout the body from any route of administration, such that any piece of skin will contain some amount of the drug. For such measurements to be useful, a similar piece of skin from another part of the body, not suspected to be an injection site, must be analyzed for comparison. Only if the concentration in the suspect site is substantially higher than that in the reference site can meaningful conclusions be drawn. Even then, a perfect injection may not cause persistent elevated drug concentrations at the intravenous injection site, in contrast to an intramuscular or subcutaneous site. Similarly, the simple detection of a drug such as cocaine in a nasal swab does not prove that the drug was "snorted." Any fluid secreted by the body, including sweat, vaginal fluid, and nasal secretions, will contain some concentration of the drug. In this instance, quantitative determination is difficult and interpretation

even more so unless the concentration of drug in the nasal secretions is extremely high relative to the blood.

6.2.2.10 Hair

Most drugs and poisons will be absorbed by bone, nails, and hair. Hair has long been used for the determination of arsenic and heavy metals, and by cutting the hair into sequential sections, for estimating the duration of exposure to the poison.²⁰ More recently, hair has been used for the determination of drugs of abuse in workplace and probation testing. Further, hair analysis can also be applied to estimate compliance in drug substitution programs and may also prove useful in therapeutic drug monitoring. In drug-facilitated crimes, the detection of a particular poison, such as GHB,²¹ zopiclone,²² and thiopental²³ in hair, has been used to document the exposure in several drug-facilitated crimes, but a negative finding can usually not exclude an exposure.²⁴ Finally, hair analysis has the potential to be useful in postmortem situations, for example, to estimate the duration of exposure to a drug or toxin, and hence provide information about the subject's previous drug use.²⁵⁻²⁹

The incorporation of drugs into hair is to a large extent due to melanin binding.³⁰ Hence, comparisons of levels between individuals is very risky. Even if the melanin content in the hair is measured, there are different types of melanin, and besides, a correction for total melanin content can only be applied to drugs where the drug-melanin binding characteristics have been firmly established. For most drugs, such information is lacking, and hence, the exact hair drug concentration per se is rarely informative.

6.2.2.11 Nails, Bone

One advantage of analysis of keratinized materials that should be emphasized is the stability of drugs in hair and nails, which means that such samples can be stored in room temperature for very long periods without major degradation of incorporated drugs. Drugs are incorporated into nails via both the root of the growing nail and via the nail bed.³¹ This implies that during the growth of the nail, drugs follow the movement of the keratinized matrix both upward and forward. In addition, the growth of nails is variable and generally slow. Hence, a temporal mapping of previous drug intake using analysis of nails is hardly possible. On the other hand, nails are almost always available for analysis, whereas hair is not: some subjects may present with alopecia totalis, or have shaved the hair on many body parts. Despite the limitations as to the growth rate of nails, this matrix has the potential to be a useful source for information about the drug use history of the decedent.

Most drugs and poisons will be taken up in bone and therefore, unless volatile, will be detectable in skeletonized remains. The interpretation of concentrations of certain drugs or poisons is relatively easy since either the normal or reference values are well established (e.g., arsenic; heavy metals), or the substance should not be present in any concentration (e.g., strychnine). However, interpretation of specific concentrations of pharmaceutical drugs or drugs of abuse is problematic because of limited reference levels. In addition, it should be recognized that bone is continuously remodeled; hence, drugs incorporated in bone tissue over time will be liberated and re-delivered to the blood. This means that a negative detection in bone does not rule out an exposure and a positive detection will not give very much information as to the time for exposure.

6.2.2.12 Paraphernalia: Syringes, Spoons, Glasses

Most forensic toxicologists are willing to analyze potentially drug-related exhibits found at the scene of death. Syringes or spoons can provide a valuable confirmation of drugs that may have been used prior to death. For example, heroin is so rapidly broken down to morphine that little or no heroin, or even monoacetylmorphine, may be detectable in postmortem blood. The finding of

morphine in, for example, blood could indicate either use of heroin or a morphine salt (or codeine, if it was also found). However, it should be borne in mind that most addicts reuse syringes and therefore the presence of a drug in a syringe found in the same room as a body does not necessarily mean that drugs contained therein were involved in the death, although it may provide circumstantial evidence. The use or abuse of insulin in a person without diabetes is exceptionally difficult to prove, since blood insulin concentrations are so variable, are difficult to determine accurately in postmortem blood, and even during life correlate poorly with blood glucose. Insulin abuse is uncommon,^{32,33} but in those cases where it happens may be difficult to prove postmortem without a good clinical history. However, detection of insulin in a used syringe near someone who was not prescribed the drug can provide useful circumstantial evidence of abuse. The presence of drug residues in drinking glasses or cups can provide evidence of at least the route of ingestion and in most cases assist with the determination of manner of death, especially if the drug residue is large and obvious. Care would obviously have to be taken to distinguish, say, a multiple drug overdose mixed in a glass of water, from two or three hypnotic tablets introduced into an alcoholic beverage for the purposes of administering a "Mickey Finn."

6.3 PHARMACOKINETICS

In this section we review the basics of pharmacokinetics as it relates to postmortem interpretation. The kinetics of all drugs and poisons in the body are characterized by absorption, distribution, metabolism, and excretion. All these parameters affect the concentrations that will be found in the body after death, and therefore interpretation of analytical toxicology results.

6.3.1 Absorption and Distribution

Absorption may be via the oral route, parenteral (e.g., intravenous, intramuscular, subcutaneous), pulmonary, dermal, and, rarely, rectal. The route of absorption can be very important to the interpretation. For example, many drugs are extremely toxic via the intravenous route, especially if given rapidly. For example, heroin, barbiturates, and many other drugs can cause severe hypotension, and may be fatal if given rapidly, even though the total dose given is within the range normally considered "therapeutic." The resulting postmortem blood concentrations may be below those normally considered fatal. At the other extreme, dermal absorption of medication is probably the slowest, such that even therapeutic concentrations in blood may take several hours to reach. Moreover, absorption of the drug may continue for several hours after the source of the drug, for example, a transdermal patch, is removed, due to the depot of medication that accumulates in the upper layers of the skin. In these circumstances the dose is difficult to control, and if toxicity occurs, it is important that the patient be monitored for several hours after the patch is removed, in case of continued toxicity.³⁴

Morphine provides a good and common example of why interpretation of blood concentrations alone in isolation from case history is difficult. First, opiate tolerance can vary tremendously between individuals and even within the same individual over a relatively short time span (days or weeks). Tolerance is an important consideration both clinically, where opiates may be chronically administered for pain, and in abuse situations where they are used for their euphoric effect. In clinical situations the issue of tolerance is complicated by the fact that patients in severe pain can tolerate higher doses of opioids than those in whom the pain is mild. It is also accepted that less opioid is required to prevent the recurrence of pain than to relieve it.³⁵ The form of the opioids will affect how rapidly the drug crosses the blood-brain barrier and, therefore, how potent it is. For example, heroin (diacetylmorphine) is at least twice as potent as morphine, probably because it is more lipid soluble and reaches the central nervous system faster than the more hydrophilic drug, morphine. It has been suggested that heroin may simply be a pro-drug for morphine, but one

that reaches the site of action more efficiently. As a result, blood concentrations of morphine seen in heroin abuse deaths are frequently lower than concentrations resulting from the therapeutic administration of oral or parenteral morphine in clinical situations. The situation is complicated further because morphine is extensively metabolized by conjugation with glucuronic acid.

Originally it was assumed that this resulted in exclusively water-soluble metabolites, which were pharmacologically inactive. However, while morphine-3-glucuronide is devoid of narcotic activity, morphine-6-glucuronide, which is typically present in blood at higher concentrations than unconjugated morphine, is more potent than morphine itself.³⁶⁻³⁸ Furthermore, much of the case data published in the clinical and forensic toxicology literature does not even distinguish between unconjugated and "total" morphine, let alone the 3- and 6-glucuronides, which are seldom measured routinely. With all these variables, it is no wonder unconjugated morphine blood concentrations correlate poorly with analgesic effect and central nervous system depression. A good example of this has been described where prolonged respiratory depression was observed in three patients in renal failure where morphine concentrations were extremely low, but where morphine-6-glucuronide had accumulated to toxic levels.³⁹

6.3.2 Metabolism and Pharmacogenetics

A detailed treatise on the mechanisms of drug metabolism and the accumulation of drugs or metabolites due to impaired metabolism is beyond the scope of this chapter. However, it is worth pointing out at least three different scenarios where impaired metabolism can have a significant impact on the interpretation of results. Metabolism can be impaired by liver disease, such as advanced cirrhosis. However, not all metabolic pathways will be impaired equally by liver disease, and indeed some pathways may be affected little, if at all. Oxidative pathways, which are easily saturable, are likely to be affected more than others, such as glucuronidation. A person's metabolism may be genetically deficient, for example, in cytochrome P4502D6 (CYP2D6). This pathway is responsible for many oxidative transformations such as ring hydroxylation of the tricyclic antidepressants, and genetically poor metabolizers can be identified postmortem.⁴⁰ Third, co-ingested drugs can inhibit one or more drug metabolism pathways. For example, most or all of the selective serotonin-reuptake inhibitors (SSRIs) inhibit CYP2D6 and some are extremely potent in this regard. The degree of elevation of the drugs or metabolites affected depends very much on the respective dosages of the drugs involved and, not least, on the "metabolic reserve" of the individual patient. Some drug-drug interactions or genetic polymorphism may only result in slightly elevated drug or metabolite concentrations, perhaps necessitating lowering of dosage. However, in some circumstances the increases may be so dramatic as to cause life-threatening toxicity or death, particularly where the side effects were not sufficiently severe to alert the physician or patient that cardiotoxicity might be a problem. At least two cases involving probable impaired metabolism of imipramine have been described in the forensic literature.⁴¹

6.3.3 Calculation of Total Body Burden

Calculation of the total amount of drug ingested in self- or homicidal poisonings has been attempted many times over the years. This was attempted by the toxicologist who analyzed the remains found in the basement of Dr. Harvey Crippen, the renowned London poisoner who used hyoscine.⁴² Calculations typically involve measurement of the drug or poison in the major organs including, where possible, skeletal muscle, and then taking into account the organ weights to arrive at a total estimate of the amount in the body. In some cases, the amounts have correlated very well with the available physical evidence (e.g., amount of drug in an empty injection vial or amount prescribed).^{43,44} Doubtless, in some other examples attempted by toxicologists, correlation with the physical evidence was less convincing, or not possible. In order for such calculations to be meaningful, a number of factors must be assumed.

Perhaps most important, the particular part of the tissue or blood sample analyzed must be representative of the remainder of the organ or tissue. Since most organs are not homogeneous and because uneven postmortem diffusion (as discussed later) can lead to non-homogeneity of concentration, being sure of the average concentration of drug within any one organ may be difficult without analyzing that entire organ. While it is easy to know the weight of individual organs such as the heart, lungs, liver, kidneys, and brain, it is very difficult to reliably estimate the total amount of tissue into which most drugs readily distribute including the skeletal muscle. While the mass of skeletal muscle can be estimated from medical tables, given a person's height and weight, there is no assurance that the concentration of drug measured in one or two portions of skeletal muscle is representative of that in muscle from all other parts of the body.

Similar arguments apply to adipose tissue, where it is more difficult to obtain representative samples and accurately assay. It should also be borne in mind that for a person chronically taking a drug with a very large volume of distribution and long half-life, the equivalent of many times the total daily dose will be *normally* present in the body, even after therapeutic doses. Estimation of the total body burden of a drug may not be without value in all cases; it must be done with caution and the variables well understood and acknowledged. It is the rare cases of homicidal poisoning where significant weight may be erroneously placed on such calculations and where the stakes are the highest.

6.3.4 Estimation of Amount Ingested from Blood Levels

Given the foregoing discussion, it should go without saying that using pharmacokinetic calculations to try to estimate dosage, given a postmortem blood concentration, is of virtually no value and can be extremely misleading. Several factors make such calculations invalid. The blood drug concentration measured postmortem must be representative of that present at the time of death. As discussed elsewhere in this chapter, that is often not the case, and it is very difficult to predict whether any given postmortem drug concentration represents the concentration at the time of death, even for drugs for which postmortem redistribution is thought to be minimal. Any toxicologist who has routinely analyzed drugs in multiple blood samples from the same case knows how often those concentrations unexpectedly vary from sample to sample. Also, the drug must be at steady state at the time the person dies. By the very nature of drug-related deaths, that is rarely the case. Even if the gastric contents contain relatively little drug, much of the drug could still be present in the ileum, or at least not have attained equilibrium with muscle, adipose tissue, and the major organs. Finally, the rate of absorption, bioavailability, volume of distribution, half-life, rate of metabolism, and clearance are seldom known for any specific individual and can vary tremendously between subjects. The estimation of dose from postmortem blood concentrations is a practice of the foolhardy.

6.4 POSTMORTEM REDISTRIBUTION AND OTHER CHANGES

One question should be asked before attempting to interpret postmortem drug concentrations: Is the concentration found likely to represent, at least approximately, that present at the time of death? Unfortunately, the answer is often a flat no, or at least not necessarily. A number of factors need to be considered.

6.4.1 Incomplete Distribution

It is often the case that sudden deaths involving drugs are caused by abuse or suicidal drug overdose. Death will therefore usually occur before steady state has been reached. If a person is actively absorbing an overdose, it is likely that the concentration of the drug in blood leaving the liver (i.e., the inferior vena cava and right atrium) will have a somewhat higher concentration than.

for example, venous blood returning from the peripheral vessels (e.g., femoral vein), for no other reason than a substantial amount of the drug will be absorbed during the course of circulation through the body. This has been demonstrated in living patients with concentration differences up to about twofold recorded between arterial and venous blood.^{45,46} It is an open question if this is a practical issue in postmortem toxicology. In two cases of almost instantaneous death following heroin injection, the concentrations of morphine and codeine in blood collected from heart, brachial veins, and femoral veins were uniform, indicating a very rapid equilibrium.⁴⁷

6.4.2 Postmortem Redistribution and Postmortem Diffusion

Postmortem redistribution and postmortem diffusion involve the movement of drug after death along a concentration gradient. Although the differentiation of these terms is not always clear in the literature, postmortem redistribution generally refers to the release of drugs from areas of higher concentration in organ tissues and subsequent diffusion into and through the capillaries and larger blood vessels of those organs. Postmortem diffusion generally refers to the diffusion of drug along a concentration gradient, from an area of high concentration to an area of low concentration. The usual scenario is where a high concentration of drug in the stomach contents (e.g., after an overdose) causes elevated concentrations of the drug in nearby tissue (e.g., proximal lobe of the liver) or blood.

Much is still unknown about the extent to which postmortem changes in drug concentration occur and the drugs affected; however, some generalizations can be made. Postmortem redistribution is likely to be most marked for drugs that are highly protein bound, but particularly those sequestered in the major organs such as the lungs and liver (e.g., tricyclic antidepressants, propoxyphene, chloroquine). Postmortem redistribution starts to occur within an hour after death and continues as the postmortem interval increases. The most important quantitative changes in blood drug concentration occur within the first 24 h and are highly site dependent. In general, increases will be greater in blood from "central" sites, such as the vessels near the major organs, than in more peripheral sites, such as the femoral veins. However, blood drug concentrations can vary fivefold or more between cardiac, hepatic, and pulmonary sites.^{2,4} Given the very close proximity of these major vessels to one another and the organs they serve, it is impossible to even estimate peri-mortem drug concentrations based on the postmortem interval and site from which a blood sample was drawn. Even aside from the unpredictable nature of postmortem redistribution per se, blood from the "heart," if labeled as such, could have come from either of the cardiac atria or ventricles, the pulmonary vein or artery, the aorta, or the inferior vena cava.

Since it is known that many drug concentrations change after death, due to redistribution from the major organs, it is recommended that postmortem blood for drug and alcohol analysis be taken from a peripheral site such as the femoral vein. However, it should be emphasized that even if a "good" femoral blood sample is obtained, it is no guarantee that the drug concentrations subsequently measured will represent those present at the moment of death. In fact it is well established that femoral blood concentrations of many drugs can increase twofold or more after death. While it is possible that some of this increase is due to diffusion of released drug down the major vessels to the groin, it should be borne in mind that drug concentrations in skeletal muscle are often twofold or more higher than in the peri-mortem blood.^{2,4} Given the mass of muscle surrounding these relatively small peripheral vessels, diffusion of drug directly into the blood across the vessel wall is very likely to occur. While in many of the published studies on postmortem redistribution the vessels have been carefully ligated prior to taking blood samples, this is rarely done during routine medicolegal autopsies. Consequently, blood labeled as "femoral" may contain blood drawn down from the inferior vena cava. This is particularly likely to be the case where large volumes (e.g., 30 to 50 mL) have been obtained from a supposedly femoral site. It should also be obvious that it does not matter whether the syringe needle is pointing down toward the leg!

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The mechanisms for postmortem redistribution probably involve release of drug from protein-bound sites after death occurs, with subsequent diffusion into interstitial fluid, through the capillaries and into the larger blood vessels. Since this process appears to start within an hour or so of death, decomposition or putrefaction per se is not likely to play a role, at least in the early stages. It is more likely that cessation of active cellular processes and the rapid fall in blood and tissue pH that occurs after death would lead to changes in the conformation of proteins and therefore release of some proportion of drugs present from the protein-bound state. It is important to bear in mind that these changes start well before putrefaction and microbiological action is likely to play a role.

Other types of postmortem diffusion can occur. For example, it has been demonstrated that over a period of a day or more, significant changes in drug concentrations in the major organs can occur. This has been shown for the tricyclic antidepressants, where concentrations in the lungs tended to decrease, commensurate with an increase in concentration in the liver.⁴⁸ This study was done in such a manner as to show that these changes can occur due to direct diffusion from one organ to the other, independent of the residue of drug in the stomach. However, the magnitude of these changes is not likely to affect interpretation of tissue drug concentrations to a significant extent. It has also been demonstrated that postmortem diffusion of drug from the stomach can markedly increase drug concentrations in proximal lobes of the liver and lungs, as well as post-mortem blood in some of the central vessels.^{12,13} Ironically, when organ tissue was analyzed in previous decades, postmortem diffusion into the liver or lungs might have been less important since it was not uncommon to homogenize large amounts of organ tissue (e.g., 500 g), such that any local increases in concentration would be averaged out. However, today the tendency in many laboratories is to homogenize small amounts of tissue (e.g., 2 to 10 g), which could lead to a gross overestimation of the amount of drug in the organ if the sampled tissue were taken close to the stomach. The potential for postmortem diffusion of drugs in this manner has been known for decades, but recent work has brought the issue the attention it deserves and better quantified the potential changes.

Aspiration of gastric contents can provide one more important mechanism whereby postmortem blood concentrations can be artificially elevated.⁴⁹ This can occur agonally, as death is occurring, or after death, during transportation of the body. It is a factor that may more commonly occur after overdosage where the stomach contains a very concentrated cocktail of one or more drugs, with or without alcohol. However, it could also be very important to consider in deaths where therapeutic doses have been consumed and death occurs as a result of unrelated natural causes. It is not uncommon, for example, for tricyclic antidepressants to be taken as a single nightly dose, and in fact large doses of many antipsychotic drugs are taken at night. This can result in drug concentrations in the stomach of the order of grams per liter, which if aspirated could result in significant increases in some local postmortem blood concentrations. Not surprisingly, the pulmonary vein and artery blood concentrations are elevated to the greatest extent following simulated aspiration. This is more significant than it might seem because much of the so-called "heart blood," which is often sampled at autopsy, is in fact blood of pulmonary origin drawn from the major pulmonary vessels or the left atrium. A comprehensive discussion of the possible mechanisms for postmortem redistribution has been published.⁵⁰

6.5 OTHER CONSIDERATIONS

6.5.1 Trauma

Severe trauma can affect the interpretation of both alcohol and drug concentrations. For example, it is not uncommon for severe motor vehicle accidents to result in rupture of the stomach and diaphragm. This can easily result in the release of gastric fluid into the body cavity. Because blood may be difficult to obtain from discrete vessels, pooled blood from the pleural cavity may

be sampled. If an autopsy is performed, the origin and nature of the fluid so drawn should be obvious, and hopefully noted. However, if an autopsy is not performed and "blood" is sampled through the chest wall in an attempt to obtain cardiac blood, the coroner or medical examiner may be unaware that the sample is contaminated with gastric fluid. If even small, therapeutic amounts of drug remain unabsorbed in the gastric contents in these circumstances, it can result in what appears to be a grossly elevated "blood" drug (or alcohol) concentration. The release of microorganisms from the gastrointestinal tract and subsequent potential for fermentation are well-recognized problems.

Trauma causing extended blood loss may also affect blood drug levels, since the physiological reactions include, in addition to increased heart rate and peripheral vasoconstriction, plasma volume refill. Hence, blood levels may increase or drop, depending on their concentrations in the restoration fluid. Experimentally, codeine and morphine blood levels were found to increase significantly after controlled exsanguination in rats⁵¹ and a similar study showed that the analgesic effect of morphine was elevated when given to rats with hemorrhagic shock.⁵² Although further studies are needed to determine the impact of and conditions for such antemortem redistribution for several drugs with different pharmacokinetic properties, the phenomenon should be considered in trauma cases with longer duration of blood loss.

6.5.2 Artifacts of Medication Delivery

Artifacts of absorption and distribution must be recognized when interpreting postmortem blood concentrations. For example, it is quite common to find grossly elevated concentrations of lidocaine in cases where resuscitation has been unsuccessfully attempted. Concentrations may be two to five times those normally considered therapeutic when lidocaine is given by intravenous infusion for the treatment of cardiac arrhythmias. If lidocaine is administered as a bolus intracardiac injection and normal cardiac rhythm never established, very high local concentrations will result in the cardiac blood. These could be interpreted as "fatal" unless all the circumstances are considered.

Devices that automatically deliver medication by the parenteral route can lead to artificially high blood concentrations postmortem. Most of these devices will continue to periodically dispense medication, usually narcotics, into the vein after a person dies, unless they are switched off and disconnected quickly. This can result in extremely high local concentrations of drug, which may be misinterpreted as an overdose.

Transdermal patches left on a body after death will give rise to locally high concentrations of the drug (e.g., fentanyl). Since these patches rely primarily on passive diffusion across a rate-limiting membrane for drug delivery, the concentration of the medication in the local area will continue to rise after death, albeit at a slower rate. Since blood circulation through the skin obviously stops after death, the drug will no longer be transported away except by diffusion, allowing a local build-up of drug. However, such a high concentration gradient exists between the gel containing the medication in the patch and the skin, that even modest postmortem diffusion might be expected to raise the postmortem blood and tissue concentrations up to several inches away.

6.5.3 Additive and Synergistic Toxicity

When interpreting drug concentrations it is important to take into account the sum of the effects of all of the drugs detected. This is often an issue in drug abuse deaths, particularly those involving prescription drugs. Such deaths often involve multiple drugs of the same type (e.g., benzodiazepines or narcotics), individually present in "therapeutic" amounts, and often in combination with alcohol. Interpretation of blood drug concentrations in these cases has to take into account disease that may be present, and the total amounts of drugs and alcohol. In many cases, these effects may simply be additive, i.e., simply the sum of the individual effects of the drugs involved. In other cases, the effect may be truly synergistic, where the toxicity is greater than would be expected based on the

pharmacology and concentrations of the individual drugs. Cases where multiple drugs are present, with or without alcohol, are probably the most difficult to interpret and rely heavily on the experience of the interpreter and a reliable and complete case history.

6.5.4 Adverse Reactions

A death attributed to neuroleptic malignant syndrome (NMS) resulting from therapy with phenothiazine or some other neuroleptics is a good example of a fatal adverse drug reaction.⁵³ Combinations of drugs can result in similar syndromes, such as combination of a tricyclic antidepressant and a monoamine oxidase inhibitor (MAOI) causing serotonin syndrome.⁵⁴ Although not always fatal, a serotonin reaction can result in death and might be considered where there is no other reasonable cause of death and especially where there are elevated concentrations of MAOIs and either tricyclic antidepressants or SSRIs. It should be borne in mind that by the very nature of drug-drug or other adverse reactions, blood concentrations of the drug(s) involved are seldom predictive of the outcome and are often well within the range normally expected from therapeutic doses. In the absence of clinical observations, such fatalities can be very difficult to diagnose accurately.

6.5.5 Drug Instability

It should not be overlooked that many drugs are unstable in any biological fluid. Cocaine is probably the most notable example. It is broken down in aqueous solution and enzymatically in blood or plasma to benzoylecgonine and methylecgonine, neither of which has much pharmacological activity. While cocaine may be stabilized to some extent by the addition of fluoride after the blood is collected, the extent of breakdown between death and autopsy must be considered. Unfortunately, there are many variables to consider. First, the toxicity of cocaine itself correlates only poorly with blood concentration, even in the living. There is good evidence that cocaine concentrations in postmortem blood can increase or decrease, depending on the exact site of collection.^{55,56} There are probably competing effects due to variable breakdown in different areas of the body and true postmortem redistribution. The collection and measurement of cocaine in vitreous humor has been attempted to overcome these problems. However, it has been shown that cocaine will often, if irreproducible, increase in concentration with time in the vitreous humor. The mechanism for this has not been proved, but it likely involves postmortem redistribution from the brain, where cocaine is known to concentrate relative to the blood, into the eye via the optic nerve and other soft tissue. It is possible that time-dependent postmortem increases in vitreous concentrations may occur for other drugs where those drugs attain higher concentrations in the brain.

6.5.6 Interpretation Using Tables of Values

There probably is not a forensic toxicologist or pathologist alive who has not used published tables as a reference when trying to interpret postmortem blood concentrations. Tables of such values became a necessary evil due to the sheer volume of medical and forensic literature. However, they unfortunately perpetuate the myth that postmortem toxicology results can be interpreted solely using, or heavily relying on, so-called "therapeutic," "toxic," and "fatal" ranges. Although tables of drug concentrations can serve as a useful reference point, it should be borne in mind that many of the values in these tables are derived from serum or plasma data from living patients, that the ranges are seldom referenced to published cases, and that they may not take into account or state other variables such as postmortem redistribution, time of survival after intoxication, or the presence of other drugs, natural disease, or injury. Having stated that, one compilation has attempted to address some of these issues and indeed bases the postmortem values it lists exclusively on carefully collected femoral blood samples.⁵⁷ In that compilation, values are also provided for "controls."

consisting of deceased subjects, who with certainty died of causes other than intoxication, and who were not incapacitated at the time for the demise. Such data are equally important as levels in fatal cases and additional compilations using this approach are encouraged.

6.6 CONCLUSION

In the final analysis, postmortem toxicology results must be interpreted with regard to all of the available information, including medical history, information from the scene, autopsy findings, nature and exact location of the postmortem samples collected, and the circumstances of the death. Only after weighing all of these variables can postmortem results be reliably interpreted. Even then, it must be admitted that *reliable* interpretation of some results is simply not possible based on the available information. In many respects, the desirable underlying approach to the interpretation of postmortem drug concentrations is not much different from that used a century ago: a good scene investigation, medical investigation, laboratory investigation, and the application of common sense. We hope we are also wiser now.

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